Thermodynamics and Kinetics of a Molecular Motor Ensemble

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ABSTRACT If, contrary to conventional models of muscle, it is assumed that molecular forces equilibrate among rather than within molecular motors, an equation of state and an expression for energy output can be obtained for a near-equilibrium, coworking ensemble of molecular motors. These equations predict clear, testable relationships between motor structure, motor biochemistry, and ensemble motor function, and we discuss these relationships in the context of various experimental studies. In this model, net work by molecular motors is performed with the relaxation of a near-equilibrium intermediate step in a motor-catalyzed reaction. The free energy available for work is localized to this step, and the rate at which this free energy is transferred to work is accelerated by the free energy of a motor-catalyzed reaction. This thermodynamic model implicitly deals with a motile cell system as a dynamic network (not a rigid lattice) of molecular motors within which the mechanochemistry of one motor influences and is influenced by the mechanochemistry of other motors in the ensemble.

INTRODUCTION

In cells, biomolecular motors often work in concert to move cargo at useful rates along long polymer tracks, and, in this paper, we develop a minimal thermodynamic model for describing this ensemble motility. A single molecular motor will move unloaded cargo along a track in nanometer (approximately motor-sized) steps (Svoboda et al., 1993; Finer et al., 1994) if, upon binding to the track, the motor deforms somewhere between its track- and cargo-binding sites (Rayment et al., 1993; Baker et al., 1998; Warshaw et al. 1998); see Fig. 1. Thermal fluctuations in motors and tracks result in a distribution of motor step sizes about an average value, d (Molloy et al., 1995). If the cargo position is fixed relative to the track, this single motor step will perform internal work on the motor–track–cargo complex, generating force in piconewton (approximately binding-energy/deformation-size) increments (Svoboda et al., 1993). Detailed calculations of this force require knowledge of how the potential energy of the motor–track–cargo complex varies with the relative positions of its constituent atoms. However, because these calculations are nontrivial for biomolecular motors, tracks, and cargo (each consisting of thousands of atoms) this potential is often approximated using a form of macromolecular mechanics in which all or part of the motor–track–cargo complex is treated as a macroscopic spring with a characteristic spring constant, k (Huxley, 1957; Hill, 1974). According to this macromolecular approximation, the average work performed by a single motor step is $\frac{1}{2}kd^2$, and the average force generated is kd.

Although macromolecular mechanics may be useful for approximating intramolecular interactions within a single motor–track–cargo complex, it alone does not account for intermolecular interactions among an ensemble of molecular motors that interact with the same track. In theory, this is not a problem for conventional independent force generator models (Huxley, 1957; Hill, 1974), because a fundamental assumption of these models is that intermolecular motor interactions are negligible (i.e., molecular motors do not feel the steps of neighboring motors). Recent studies, however, challenge the validity of this assumption (Leibler and Huse, 1993; Baker et al., 1999; Baker and Thomas, 2000), and so, in this paper, we describe the mechanochemistry of an ensemble of molecular motors by applying solution thermodynamics to the two-state model defined in Fig. 1. The thermodynamic model presented below implicitly considers intermolecular motor interactions and is thus consistent with existing ensemble motor theories (Jülicher and Prost, 1995; Vilfan et al., 1998), in which intermolecular interactions are explicitly considered.

MODEL

In our model, a motor fixed to cargo deforms upon binding a track, resulting in an average motor step size, d (Fig. 1). We consider an equilibrium system of noninteracting (ideal) motors that reversibly bind to a track at constant temperature and pressure. The free energy for the motor–track binding reaction is $\Delta G = \Delta G^\circ + RT \ln(n_{MT} \Pi_i n_i / n_M \Pi_j n_j)$, where ΔG° is the standard reaction free energy, and n_{MT} and n_M are the equilibrium concentrations of motors in the MT and M states, respectively. In this equation, the products of ligand concentrations, n_i and n_j, are taken over all ligand species, i, released and all ligand species, j, bound with an M → MT transition, and all concentrations are defined as having molar units. We assume that a constant force, F, applied to a track against an ensemble of motors rapidly equilibrates among all $n = n_M + n_{MT}$ motors (Baker et al., 1999), and we define positive force to be in the direction that opposes an M → MT step. If a track moves in a

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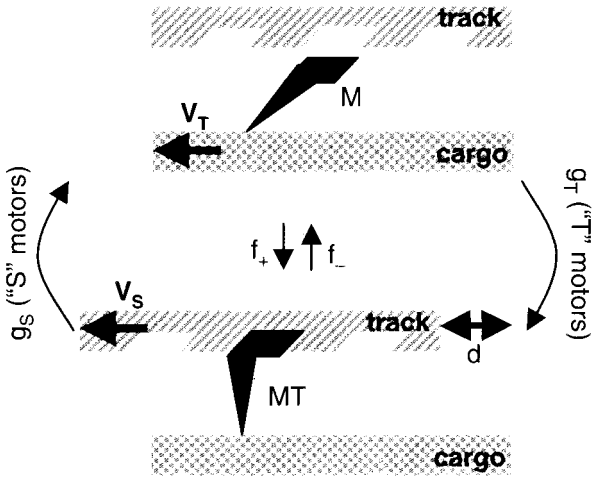

FIGURE 1 A two-state molecular motor model. A motor (*black*) fixed to cargo (*cross-hatched*) deforms (shown here as a bend) upon binding a track (*hatched*), moving its cargo a distance, d, along the track. We assume that motors equilibrate with the track, binding to and detaching from the track (*vertical arrows*) with first-order rate constants, f_+ and f_-. Net movement can be achieved by perturbing the binding equilibrium through one of two nonequilibrium processes (*curved arrows*): S motors detach from the track (at an effective rate g_S) and T motors attach to the track (at an effective rate g_T) through processes in which no work is performed on or by the motor. Upon relaxation of the binding equilibrium, S motors move the track at a velocity V_S with respect to the cargo, and T motors move the cargo at a velocity V_T with respect to the track. The unidirectional curved arrows illustrating the nonequilibrium processes indicate the direction of a net motor flux and not necessarily the irreversibility of the process.

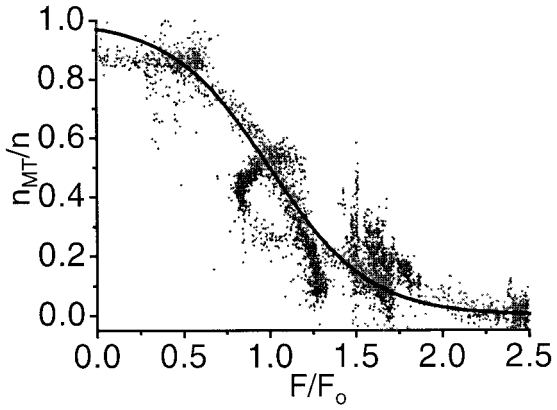

FIGURE 2 Test of the motor equation of state, Eq. 1, (*solid line*) against measurements (*dots*) of n_{MT} versus F in muscle. The M and MT states of spin-labeled myosin motors in muscle have characteristic electron paramagnetic resonance (EPR) lines, and the relative intensities of these lines provide a direct measure of the fraction of motors in the M and MT states (Baker et al. 1998). A spin-labeled regulatory light chain (RLC) from chicken gizzard myosin was labeled with a cysteine-specific spin probe and functionally exchanged with the native RLC in scallop adductor muscle, as previously described (Baker et al., 1998). The low-field peak of the X-band EPR spectrum of these fibers was monitored, as previously described (Baker et al., 1998), at 25°C during continuous perfusion with a pH 8.0 buffer containing 5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM NaN_3, 5 mM MgADP, 2 mM vanadate (V_i), 0.1 mM AP5A, and 60 units hexokinase. Hexokinase and AP5A were added to deplete contaminating MgATP. Muscle force, F, measurements were made during EPR signal acquisitions using a strain gauge mounted to the side of the EPR cavity (Baker et al., 1999). The muscle force was slowly increased at a rate of 0.001 F_0 sec^{-1} by increasing the buffer perfusion rate. Muscle-force data has been plotted against normalized EPR data (*dots*), and fitted to Eq. 1 (*solid line*) using $\Delta\mu_l = F_0 d/n = 3.5RT$ in accord with Eq. 2. Assuming $d = 10$ nm and $n = 1.0 \times 10^{-13}$ moles·mm^{-2}·half-sarcomere^{-1} this value of μ_l corresponds to an active isometric muscle force, F_0, of approximately 7 N·cm^{-2}.

direction opposite an M → MT step with a force, F, applied to the track, work is performed on the motor ensemble. The work required to shift one mole of motors from MT to M is $W' = -Fd/n$, where the direction of an M → MT step is defined as positive. This is often referred to as reversible work. It is the work performed on motors when one mole of motors undergoes a reverse MT → M step and is the work performed by motors when one mole of motors undergoes an M → MT step. At equilibrium, no net work is performed and $\Delta G = W'$ (Kittel and Kroemer, 1980), or

$$RT \ln(n_{MT}/n_M) = -\Delta\mu_l - Fd/n, \quad (1)$$

where $\Delta\mu_l \equiv \Delta G° + RT \ln(\Pi_i n_i/\Pi_j n_j)$. At equilibrium, the forward, $f_+ n_M$, and reverse, $f_- n_{MT}$, rates for the binding reaction are equal, or $f_+ n_M = f_- n_{MT}$, where f_+ and f_- are the first-order rate constants for binding and detachment, respectively (Fig. 1). In Fig. 2 we show that Eq. 1 accurately describes the observed force-dependence of a motor–track binding equilibrium in muscle. A motor–track binding equilibrium, however, cannot perform net work; for this, the equilibrium must be chemically perturbed.

The motor–track binding equilibrium can be perturbed by either attaching or detaching motors from the track through a nonequilibrium process that neither performs nor absorbs work (Fig. 1, *curved arrows*). The nonequilibrium detachment process takes a motor through a pathway in which the motor reverses its stepping motion (unbending in Fig. 1) only after detaching from the track, perturbing the binding equilibrium with a net transfer of motors from MT to M. We refer to motors that undergo this process as S motors. The nonequilibrium attachment process takes a motor through a pathway in which the motor induces its stepping motion (bending in Fig. 1) before attaching to the track, perturbing the equilibrium with a net transfer of motors from M to MT. We refer to motors that undergo this process as T motors. Either nonequilibrium process could be achieved through a motor-catalyzed chemical reaction, such as the adenosine triphosphatase reaction. In this paper, we assume that both nonequilibrium processes are quasi-static; i.e., in both cases, the effective rate, g_S or g_T, of the catalyzed reaction is much slower than the relaxation rate, $f_+ + f_-$, of the binding equilibrium.

In Fig. 1 (*curved arrows*), we illustrate each nonequilibrium process with a single chemical step having an effective rate, g_S or g_T, where $g_S n_{MT}$ and $g_T n_M$ are the net motor fluxes through the S and T motor pathways, respectively.

Intermediate steps and rates involved in these processes differ among motors and can be considered on a case by case basis (e.g., Baker and Thomas, 2000). The unidirectional arrows used to illustrate these processes (Fig. 1) indicate the direction of the net motor flux and not necessarily the irreversibility of the process.

The motor-catalyzed "reaction cycle" is completed with the relaxation of the motor–track binding equilibrium (M $\leftrightarrow$ MT). When a motor–track binding equilibrium relaxes following an MT $\rightarrow$ M perturbation, motors move the track (V_S in Fig. 1) relative to the cargo in the direction of an M $\rightarrow$ MT *step*; hence the S motor designation. When a motor–track binding equilibrium relaxes following an M $\rightarrow$ MT perturbation, motors move cargo (V_T in Fig. 1) relative to the track in the direction of an MT $\rightarrow$ M *throw* (a reverse step); hence the T motor designation. We refer to the relaxing step(s) of an S motor and the relaxing throw(s) of a T motor as working strokes (note that the T motor working stroke is a throw, not a step).

In Fig. 3, we describe kinetic analogies for these two motor classes. Similar to the model in Fig. 1, molecular motors in Fig. 3 perform work on a wheel upon binding to the wheel, and the wheel performs work on molecular motors upon detachment. Moreover, in Fig. 3, as in the Fig. 1 model, a binding equilibrium is perturbed through one of two nonequilibrium processes (*curved arrows*): An S motor detaches from the wheel, and a T motor attaches to the wheel through pathways in which work is performed neither on nor by the motor. With the subsequent relaxation of the binding equilibrium, S motors perform net work and net work is performed on T motors in what we refer to as a working stroke. An ensemble of S motors turns the wheel in the direction (V_S in Fig. 3) of a step (or collision), whereas an ensemble of T motors turns the wheel (V_T in Fig. 3) in the opposite direction. Figure 3 illustrates how the free energy for the nonequilibrium process that perturbs the binding equilibrium is not necessarily localized to a single molecule but may be inextricably mixed up among the turning wheel and all of the molecules with which it interacts.

By definition, a quasi-static process occurs without significantly perturbing the parameters in Eq. 1 from their near-equilibrium values. When one mole of motors is slowly transferred between M and MT through a quasi-static process, the equilibrium free energy of the system is perturbed by $RT\ln(n_{MT}/n_M)$ (Eq. 1), and this free energy is then available for work with the subsequent rapid relaxation of the motor-track binding equilibrium. Thus, when $RT\ln(n_{MT}/n_M) = 0$ (or $Fd = -n\Delta\mu_l$, Eq. 1), no free energy is available for work, and cargo movement along the track stalls at a force

$$F_0 = -n\Delta\mu_l/d. \quad (2)$$

According to a macromolecular mechanics model in which individual cargo–motor–track complexes are treated as linear springs with spring constant k, the average force generated, kd, by a single motor step against a fixed track is proportional to the motor step size, d. Eq. 2 predicts the inverse relationship for an ensemble of motors: The maximum force generated by an ensemble of motors is inversely proportional to d. Eq. 2 also predicts that stalling forces vary as the log of the concentration of ligands bound or released with the working stroke as observed with myosin motors (Cooke and Pate, 1985; Baker and Thomas, 2000). Moreover, our model predicts that, when stalled, the distribution of motors ($n_{MT} = n_M$) is robust with respect to changes in ligand concentrations and force as observed in stalled active muscle (Baker et al., 1999).

The free energy available for work, $RT\ln(n_{MT}/n_M)$, can be rewritten in terms of Eqs. 1 and 2 as $RT\ln(n_{MT}/n_M) = (F_0 - F)d/n$ (or if positive force is redefined to be in the direction generated by T motors—the direction of an MT$\rightarrow$M throw—$RT\ln(n_M/n_{MT}) = (F_0 - F)d/n$). We argue

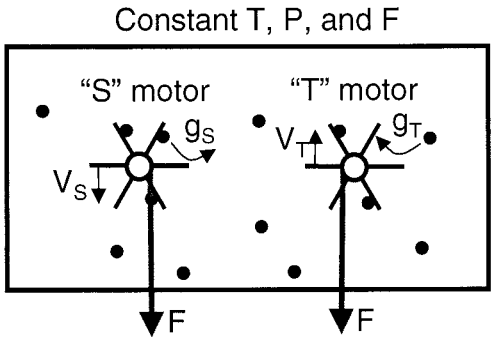

FIGURE 3 A two-state kinetic model of molecular motors. We consider an equilibrium system of noninteracting molecules at constant temperature, T, and pressure, P, that can bind to specific sites located on one side of the blades of a paddle wheel. At equilibrium, the wheel does not turn. However, if molecules are detached from these sites at a rate g_S (S motors) or if molecules are attached to these sites at a rate g_T (T motors) through processes that neither perform nor absorb work, S molecules will subsequently perform net work on the wheel or the wheel will subsequently perform net work on T molecules upon relaxation of the binding equilibrium. In both cases, the relaxation of the binding equilibrium can cause the wheel to turn at a velocity, V, against an external force, F; and the moving wheel, in turn, can bias net work production by spontaneous binding transitions as follows. With each spontaneous attachment/detachment, the turning wheel decreases S molecule velocities and increases T molecule velocities. The kinetic energy lost with spontaneous S molecule binding transitions is lost to the moving wheel as work and heat, and the kinetic energy gained with spontaneous T binding transitions could, in theory, be used to perform work on a neighboring motor, if motors were tethered together. Thus, for both classes of molecular motors, net work is performed with each spontaneous binding transition. We emphasize that this is not a thermal ratchet model in that a thermally equilibrated motor (or pawl) does not ratchet the wheel to turn unidirectionally. This is also not a Maxwell's demon model in that there is no mechanism in the wheel that decides when to let it turn. The wheel turns because a nonequilibrium chemical reaction perturbs the binding equilibrium in one direction. We present this model as a simple illustration of how the free energy for this nonequilibrium reaction is inextricably mixed up among the turning wheel and all of the molecules that interact with it.

below that the rate at which $(F_0 - F)d/n$ is transferred to work, w, and heat, q, is the forward working stroke rate ($b = f_+n_M$ for S motors and $b = f_-n_{MT}$ for T motors), and thus the net free energy output from motor working strokes is

$$\frac{dw}{dt} + \frac{dq}{dt} = \frac{b(F_0 - F)d}{n}. \quad (3)$$

According to this equation, the free energy available for work, $(F_0 - F)d/n$, is localized to a single step (the motor–track binding step) in the motor-catalyzed reaction cycle, whereas the free energy for the rest of the cycle is used to accelerate the rate, b, at which $(F_0 - F)d/n$ is transferred to work. Although $(F_0 - F)d/n$ may be only a fraction of the ATP hydrolysis enthalpy, ΔH_{ATP}, we suggest that the free energy output from motor working strokes (left side of Eq. 3) can approach the ATP hydrolysis enthalpy output ($g_S n_{MT}\Delta H_{ATP}$ for S motors) if the free energy for ATP hydrolysis is used to accelerate multiple working strokes per ATP hydrolyzed (e.g., $b > g_S n_{MT}$ for S motors). Conservation of energy provides an energetic limit for the number of working strokes per ATP hydrolyzed (e.g., $b/g_S n_{MT} \leq n\Delta H_{ATP}/(F_0 - F)d$ for S motors) and shows that multiple working strokes per ATP hydrolyzed are energetically feasible if $(F_0 - F)d/n < \Delta H_{ATP}$. We suggest that multiple working strokes per ATP hydrolyzed are achieved when cargo–track movement prevents reverse S motor steps from performing negative work, forcibly detaches motors, or throws T motors with a greater internal force (see kinetic analogies in Fig. 3). In these ways, cargo–track movement can accelerate the free energy transfer rate, b, beyond the rate, $g_S n_{MT}$ or $g_T n_M$, of the non-equilibrium chemical process.

If motors move their cargo a distance x along a track against a constant force, F, the net motor power output, dw/dt, is $F(dx/dt) = FV$, where V is the velocity of the cargo with respect to the track. Here the external work, Fx, is irreversible and is quite different from the reversible work, Fd/n. Specifically, external work is lost in moving an external load, whereas reversible work is both performed by motors on compliant elements (with steps) and performed by compliant elements on motors (with reverse steps). In fact, Eq. 3 shows that the reversible work takes away from the free energy available for external work, and, when the reversible work is at a maximum ($Fd/n = F_0d/n$), the external work is zero. If motors move their cargo a distance x along a track in the direction of a given working stroke, the working stroke step or throw size from the reference frame of the moving track is $d - x$. The remaining fraction, x/d, of the total free energy available for work, F_0d/n (Eq. 2), by working strokes is lost as heat to cargo–track movement (analogous to the heat of expansion for a gas). At a given time, only a fraction, a, of motors are undergoing working strokes, and so the heat output for cargo–track movement is $dq/dt = aF_0(dx/dt) = aF_oV$, where a is the motor duty ratio. Substituting the above expressions into Eq. 3, we obtain the motor force–velocity relationship,

$$V = (bd/n)(1 - F/F_0)/(F/F_0 + a). \quad (4)$$

This equation is analogous to Hill's (1938) semi-empirical force–velocity relationship for muscle, and, in Fig. 4, we plot this equation for three different values of a. Figure 4 shows that, with all other parameters constant, low-duty ratio motors ($a < 1$) move their cargo along a track at higher velocities than high-duty ratio motors ($a \approx 1$). This is because velocities of low-duty ratio motors result from consecutive, rapid intermediate working strokes of multiple ($1/a$) motors in the ensemble, whereas velocities of high-duty ratio motors are determined by rate-limiting working strokes of a smaller number of motors in the ensemble. This would further suggest that $1/a$ is related to the number of motors required for an ensemble velocity, V, which is the minimum number of motors to which our ensemble theory can be applied.

DISCUSSION

We have defined a motor step as a discrete motor bending motion that occurs upon track binding ($M \rightarrow MT$ via straight arrow in Fig. 1) and a motor throw as the reversal of this bending motion that occurs upon track detachment ($MT \rightarrow M$ via straight arrow in Fig. 1). If molecular forces rapidly equilibrate among an ensemble of motors, the force dependence of a motor–track binding equilibrium is described by Eq. 1. A motor–track binding equilibrium can be perturbed through one of two quasi-static, nonequilibrium, chemical processes (Fig. 1, *curved arrows*), each characterizing a distinct motor class. The nonequilibrium S motor

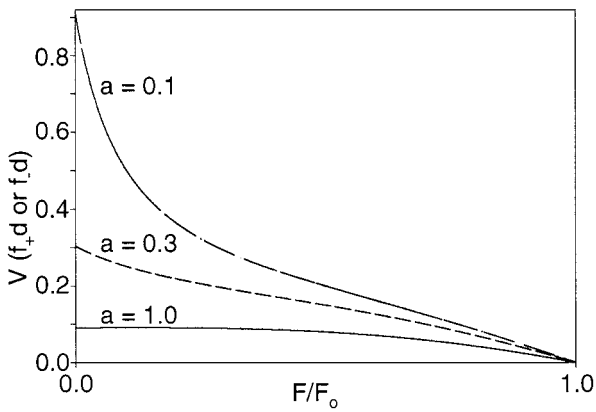

FIGURE 4 The motor force–velocity equation (Eq. 4) plotted for three different values of a. Here we assume that f_+ is constant for S motors and that f_- is constant for T motors, and we obtain b ($f_+ nM$ for S motors and f_-n_{MT} for T motors) from Eq. 1, setting $\Delta\mu_1 = F_od/n = 2.3RT$. The units of velocity are f_+d for S motors or f_-d for T motors. For $a = 0.1$, the curve accurately describes (Baker and Thomas, 2000) observed (Hill, 1938) force–velocity relationships of the highly cooperative myosin motors in muscle.

process takes a motor through a pathway in which the motor detaches from the track and then undergoes an unbending motion. The nonequilibrium T motor process takes a motor through a pathway in which the motor undergoes a bending motion and then attaches to the track. A *working stroke* is the relaxation response to this chemical perturbation. The S motor working stroke is a step, and the T motor working stroke is a throw. In essence, S motors in an ensemble step their cargo along a track, whereas T motors in an ensemble throw their cargo along a track.

The pathways defined above for S and T motors can be used to classify biomolecular motor mechanisms. For example, a detachment of a muscle myosin motor from actin induced by ATP binding and followed by the reversal of a myosin light chain domain rotation (Lymn and Taylor, 1971; Baker et al., 1998) would be classified as an S motor mechanism. A rotation of a kinesin motor's neck-linker (Rice et al., 1999) induced by ATP binding and followed by microtubule binding would be classified as a T motor mechanism.

Moreover, the model predictions for S and T motors can be used to classify biomolecular motor biochemistry and ensemble function. We have shown that an ensemble of S and T motors move cargo in opposite directions; they generate force in opposite directions; they have opposing track-binding biochemistry (opposite $\Delta\mu_l$); and they have stalling forces with opposite ligand dependencies (Eq. 2). These differences between two theoretical motor classes resemble the characteristic differences observed between muscle myosins and conventional kinesin motors (Cooke and Pate, 1985; Vale et al., 1985; Romberg and Vale, 1993). However, two S motors that differ both in their bend directionality and in their track binding biochemistry might also exhibit these differences, and so, to determine whether kinesin (or any motor) functions as a T motor, we must consider other distinguishing features of T motors. First, our model predicts that the direction of a T motor step upon track binding is opposite the direction of its working stroke (or throw). Thus, the direction that a single T motor step moves a track held by a bead in a laser trap should be opposite the direction that an ensemble of T motors moves a track in an in vitro motility assay. Second, our model predicts that, when force is increased (in the direction generated by the ensemble of motors), the fraction of attached S motors decreases ($RT\ \ln(n_{MT}/n_M) = (F_0 - F)d/n$) as shown in Fig. 2, whereas the fraction of attached T motors increases ($RT \ln(n_M/n_{MT}) = (F_0 - F)d/\mathrm{n}$). This may explain why, at sufficiently high forces, negative velocities are unrestricted by myosin motors in muscle (Harry et al., 1990), whereas negative velocities are prevented by kinesin motors (Coppin et al., 1997).

In our model, a motor undergoes a discrete structural change upon track binding. If a track is fixed relative to the cargo, force generation is localized to this step. If either the track or cargo is unloaded, motion generation is localized to this step. If either the track or cargo is allowed to move against a constant load, external work and free energy transfer are localized to this step. Although the free energy available for work is localized to this step, the rate, b, at which this free energy is transferred to work is accelerated both chemically and mechanically by the free energy for the nonequilibrium reaction.

In general, our model implicitly considers complex relationships between force, kinetics, and energetics in an ensemble of molecular motors. Molecular forces equilibrate among motors in an ensemble; the internal work performed by one motor can cooperatively accelerate the energy-transfer rate of other motors in an ensemble; a force applied to an ensemble of motors affects both the free energy, $(F_0 - F)d/n$, available for work and the rate, b, at which this energy is transferred to work. This level of complexity is rarely considered when describing the behavior of a molecular motor ensemble; if this complexity does exist, the thermodynamic model presented in this paper may provide important guidelines for more explicit molecular models.

CONCLUSION

Any molecule that deforms upon ligand binding functions as a molecular motor by moving the ligand with respect to a distal part of the molecule (Fig. 1). The challenge in molecular motor design is to find a way to repeat these binding events, or motor steps. One approach would be to tightly couple a motor step to a nonequilibrium step in a motor-catalyzed reaction, but, with this approach, most of the reaction free energy would be used on a single working stroke regardless of how much work is performed. We suggest that some biomolecular motors have evolved to take advantage of a different, cooperative strategy for improving motor efficiency. A binding equilibrium can be perturbed through one of two nonequilibrium processes (Fig. 1, *curved arrows*), each resulting in a distinct motor class. The subsequent relaxation of the binding equilibrium can drive net cargo–track movement (Fig. 1), which in turn can bias net work production by other motors in the ensemble (see kinetic analogy in Fig. 3). Thus, the free energy from the nonequilibrium process (e.g., the adenosine triphosphatase reaction) need not be localized to a single motor step or even to a single motor, but may be distributed among the moving cargo–track and all motors that interact with the cargo and track. In this way, the free energy from ATP hydrolysis can be distributed over multiple working strokes of multiple motors, maximizing the efficiency of motors that work together in moving their cargo along a track.

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